

DECLARATION

I declare that I am PROFESSOR ROBERT BROWN, Director of Laboratory Research Medical Oncology of CR-UK Beatson Laboratories, Gartcube Estate, Glasgow, G61 1BD, UK.

I furthermore declare that I was tasked by the assignee, Prolifix Limited, of US Patent Application Serial Number 09/900,147 in the name of La Thangue et al., and filed on July 9, 2001, to design and oversee an experiment to determine the effects of E2F peptides *in vivo* on tumour take, the results of which experiment address the objections by Examiner Yu in the Office Action of February 23, 2004 under 35 USC s112 to the failure of claims 30 and 31 currently on file to comply with the enablement requirements by demonstrating using an *in vivo* model that the polypeptide of the invention is effective in the inhibition of tumour take.

The experiment and the results obtained were performed as follows:

Effects of E2F peptides on tumour take.

To investigate whether the E2F peptides have an effect on *in vivo* tumour take in a mouse model, the following experiments were performed. A2780 ovarian cancer cells were exposed to H2* peptide (antennapedia tagged SEQ ID NO:3) (working solution of 30 µM) and rotated for 3hrs at 37 °C before being injected subcutaneously into the flanks of athymic nude mice. The antennapedia uptake sequence alone (ANT) and the uptake sequence linked to a scrambled H2 sequence (AHS2) were used as negative controls. Cells were injected at a concentration of 10⁶ cells in 100 µl per injection site, two sites per mouse. Tumour number and size were recorded at day 10.

Results:

<u>Treatment</u>	<u>Average tumour size</u>	<u>Tumour formation</u>
ANT	8x12mm	12/12
AHS2	8x9mm	12/12
H2*	6x6mm	2/12


To examine the effect of cell number on tumour formation, cells were exposed to peptide as previously described and injected into mice at a concentration of 10^5 cells per injection site. Tumour size and number were recorded at day 15. There were five mice in each of two groups.

Results:

<u>Treatment</u>	<u>Average tumour size</u>	<u>Tumour formation</u>
AHS2	8x9mm	7/10
H2 ^a	6x8mm	3/10

Thus it is apparent that the peptide specifically inhibits tumour take in this mouse model, at 10^5 and 10^7 tumour cells injected.

Signed



Witnessed



Date

22/4/04